

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2004/000493

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl. ⁷ : A01H 5/00, C12N 15/29 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPIDS, CA, MEDLINE, AGRICOLA: phosphoenolpyruvate carboxylase, PEPC, malate dchydrogenase, MDH, plsmt, grass, rye grass, lolium, fescue, festuca, clover, trifolium, medic, medicago, transgenic, transorm, genetic engineer, genetic modify, krebs, TCA, organic acid, soil		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Beaujean A <i>et al</i> , "Integration and expression of Sorghum C4 phosphoenolpyruvate carboxylase and chloroplastic NADP+-malate dehydrogenase separately or together in C3 potato plants", <i>Plant Science</i> , 2001, 160:1199-1210 whole of document	9, 18
X	Gallardo F <i>et al</i> , "Monocotyledonous C4 NADP+-malate dehydrogenase is efficiently synthesized, targeted to chloroplasts and processed to an active form in transgenic plants of the C3 dicotyledon tobacco", <i>Planta</i> , 1995, 197:324-332 see page 331, right column, paragraph beginning line 15	9, 18
X	WO 2000/073475 A1 (Pioneer Hi-Bred International) 7 December 2000 page 4 line 16 to 23	9, 18
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
Date of the actual completion of the international search 18 June 2004	Date of mailing of the international search report 24 JUN 2004	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. (02) 6285 3929	Authorized officer GARETH COOK Telephone No : (02) 6283 2541	

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	Samac DA <i>et al</i> , "Plant improvement for tolerance to aluminum in acid soils - a review", <i>Plant Cell, Tissue and Organ Culture</i> , December 2003, 75(3):189-207 see pages 202-203	9, 18
E, X	US 2004/116682 A1 (Cheikh <i>et al</i>) 17 June 2004 whole of document	9, 18
A	Häusler RE <i>et al</i> , "Single and double overexpression of C4-cycle genes had differential effects on the pattern of endogenous enzymes, attenuation of photorespiration and on contents of UV protectants in transgenic potato and tobacco plants", <i>Journal of Experimental Botany</i> , 2001, 52(362):1785-1803 whole of document	
A	Häusler RE <i>et al</i> , "Overexpression of C4-cycle enzymes in transgenic C3 plants to improve C3-photosynthesis", <i>Journal of Experimental Botany</i> , 2002, 53(369):591-607 whole of document	
A	Tesfaye M <i>et al</i> , "Overexpression of Malate Dehydrogenase in Transgenic Alfalfa Enhances Organic Acid Synthesis and Confers Tolerance to Aluminum", <i>Plant Physiology</i> , 2001, 127:1836-1844 whole of document	
A	EP 1 122 316 A1 (Centro de Investigacion y Estudios Avanzados del Instituto nacional Irapuato) 8 August 2001 whole of document	

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See supplemental sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 9, 10, 18, 19 as requested by the Applicant.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

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Supplemental Box

(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No III: Observations where unity of invention is lacking.

The problem addressed by the current application is the modification of organic acid biosynthesis in plants. The solution is provided through the use of polypeptides involved in the organic acid biosynthesis, and their encoding polynucleotides, from clover (*Trifolium*), medic (*Medicago*), ryegrass (*Lolium*) or fescue (*Festuca*), specifically polypeptides and their encoding polynucleotides from white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*). These polypeptides have been placed into three broad groupings by the Applicant: malate dehydrogenases (MDH), citrate synthases (CS) and phosphoenol pyruvate carboxylases (PEPC).

The general concept underlying the application appears to reside in enzymes involved in organic acid biosynthesis. However the enzymes involved in the organic acid biosynthetic pathway through the tricarboxylic acid cycle (TCA) are known in the prior art, as has been admitted by the Applicant page 2 lines 18 to 22 of the specification. Therefore the involvement of the enzymes in organic acid biosynthesis cannot be considered a special technical feature. The enzymes themselves may be from clover, medic, ryegrass or fescue, with the specifically disclosed sequences being from either from white clover or perennial ryegrass. However the species of origin can only constitute a special technical feature if the species of origin makes a contribution over the prior art. There is nothing in the application to indicate that isolation of peptides from white clover makes an inventive contribution over the prior art, therefore the species of origin cannot be considered a special technical feature.

Since there is no obvious special technical feature, it is appropriate to use the Markush approach to analyse the application for unity of invention.

(A) The common property is the involvement of the enzymes in the organic acid biosynthesis in plants.

(B) (1) There is no common structure that is a significant structural element shared by all the polypeptides. A significant structural element is one that forms the contribution of the polypeptides over the prior art, and is disclosed in the application.

(B) (2) There is no single recognised class of compounds embracing all the polypeptides, as the polypeptides belong to different classes of proteins, ie. MDH, CS and PEPC, each carrying out different biological functions.

Unity of invention is therefore lacking in the application.

The Applicant has placed the enzymes into three groups: MDH, CS and PEPC. Each of these groups needs to be analysed to determine if there is unity within the Applicant's groupings. Taking MDH, this group of enzymes is known in the prior art, as has been admitted by the Applicant at page 2 lines 18 to 22 of the specification. As this is a known grouping, Markush practice again needs to be applied to determine if unity exists.

(A) The common property is the involvement of the MDHs in the reversible conversion of malate to oxaloacetate.

(B) (1) There is no common structure that is a significant structural element shared by all the MDH enzymes that has been disclosed in the specification.

(B) (2) There is no single recognised class embracing all MDHs, the recognised class being one where there is an expectation that all members of the class will behave in the same way in the context of the claimed invention. According to the application in the paragraph bridging pages 2 and 3, MDH is important in several metabolic pathways and plants contain multiple forms that differ in coenzyme specificity and subcellular location. The diversity of function of MDHs is also reflected in that there are multiple enzyme classification (EC) numbers into which the enzymes are placed according to function, the numbers being 1.1.1.37, 1.1.1.38, 1.1.1.39, 1.1.1.40, 1.1.1.82 and 1.1.1.83.

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Supplemental Box

(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No III: Observations where unity of invention is lacking.

Unity in the Applicant's grouping of MDH enzymes is therefore lacking. Each polypeptide sequence within the Applicant's MDH grouping is considered to be a separate invention. Similarly with PEPC each polypeptide sequence is considered a separate invention as no significant structural element has been identified in the application, and there is no single recognised class embracing all PEPCs - the application states at page 3 lines 4 to 10 that PEPCs are widely distributed through most plant tissues filling various physiological roles, and these enzymes have different EC numbers depending on their coenzymes, the EC numbers being 4.1.1.31, 4.1.1.32, 4.1.1.49. The CS group is considered to be a single group for the purposes of unity, there being only one class embracing all CS enzymes.

The application is therefore considered to be to 37 separate inventions. The 37 separate inventions are:

1. citrate synthases (CS)

2 to 37. each separate polypeptide sequence of the Applicant's groupings MDH and PEPC (ie. each polypeptide of SEQ IDs 22, 31, 35, 37, 39, 41, 45, 47, 112, 114, 116, 184, 186, 188, 190, 198, 200, 202, 204, 206, 219, 253, 272, 277, 289, 294, 297, 303, 307, 309, 311, 316, 320, 324, 326 and 348 is a separate invention).

The Applicant requested the search be limited to claims 9, 10, 18 and 19. Claims 9 and 10 are directed to a construct comprising sequences encoding MDH, PEPC and optionally CS. Claims 18 and 19 are directed to a method of modifying organic acid synthesis by transforming a plant with sequences encoding MDH, PEPC and optionally CS. Both a construct comprising sequences encoding MDH and PEPC and a plant transformed with such a construct are disclosed in:

Beaujean A et al, "Integration and expression of Sorghum C4 phosphoenolpyruvate carboxylase and chloroplastic NADP+-malate dehydrogenase separately or together in C3 potato plants", *Plant Science*, 2001, 160:1199-1210

Hence these claims lack unity, *a posteriori*.

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Information on patent family members

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This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	200/073475	AU	51593/00	CA	2 361 912	US	6 653 535
		BR	0010975	EP	1 181 380	US	2004/078839
EP	1 122 316	AU	45533/98	BR	9815878	WO	1999/063100

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX